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Cantilever-based Force Spectroscopy for Chemical and Biological Detection

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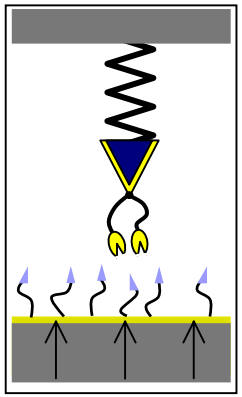
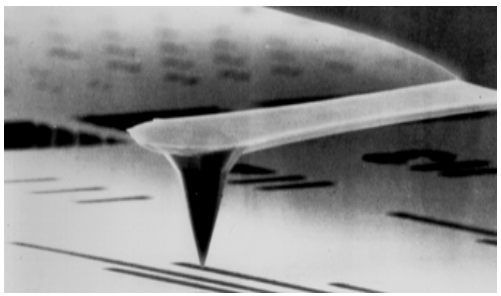
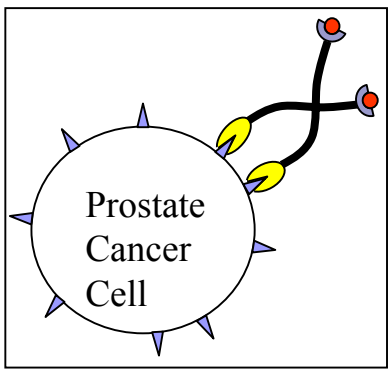
May 18, 2004 through May 19, 2004







Cantilever-based Force Spectroscopy for Chemical and Biological Detection

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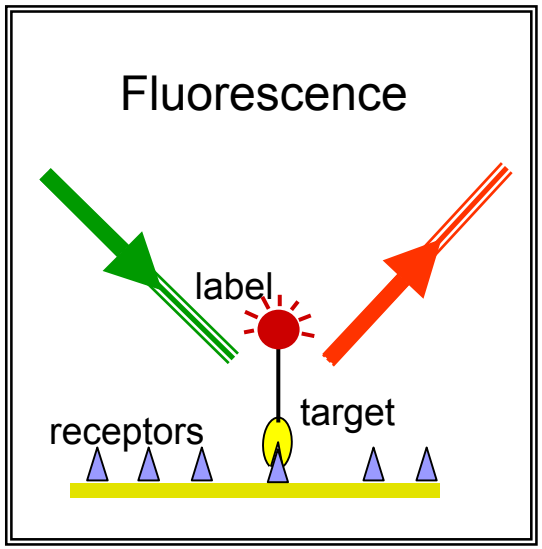
Mike Colvin, LLNL

Sally DeNardo, U.C. Davis

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract Number W-7405-Eng-48.

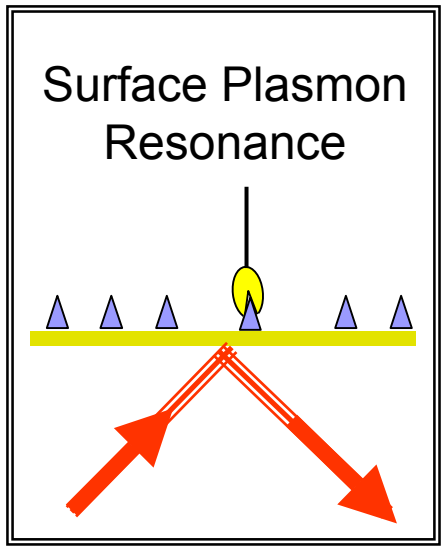
How may we identify whether a target is bound to a receptor



-Yes/no indicator

-requires labeling

-limited number of targets detected in parallel

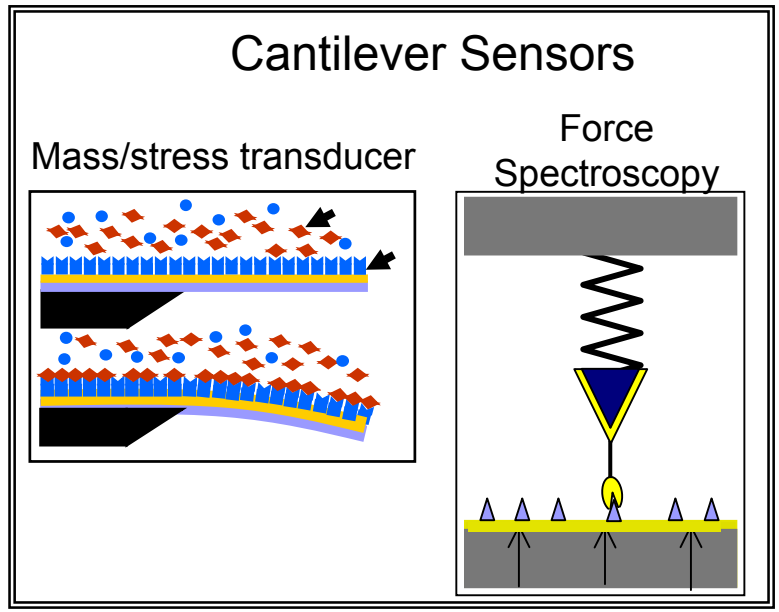


-easily quantified affinity

-real-time binding

-copious target required

-*in vitro* only



-*in vivo* measurements

-parallel target detection

-small target amount

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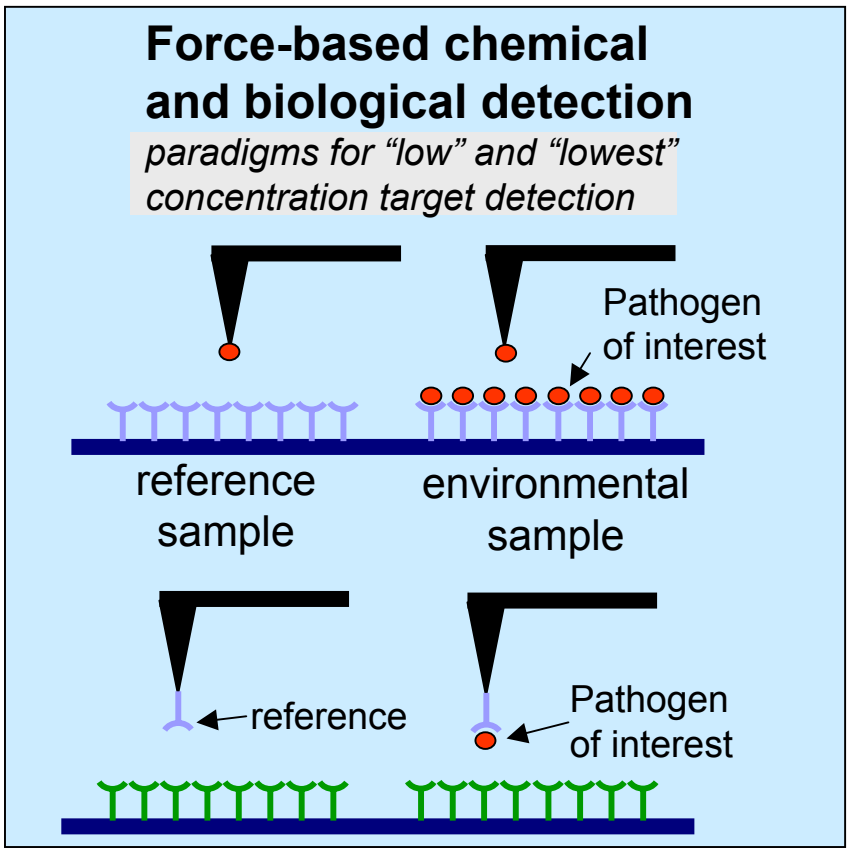
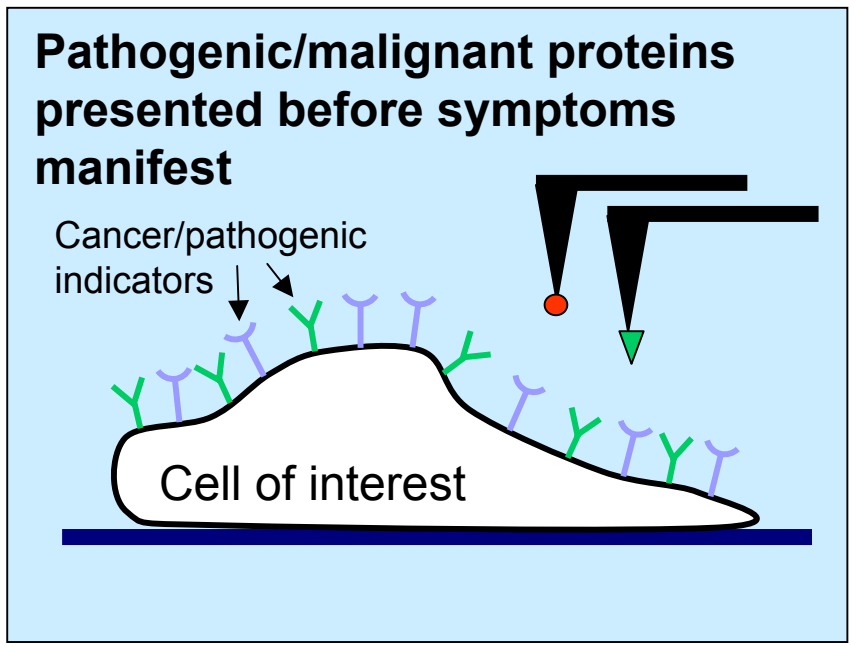
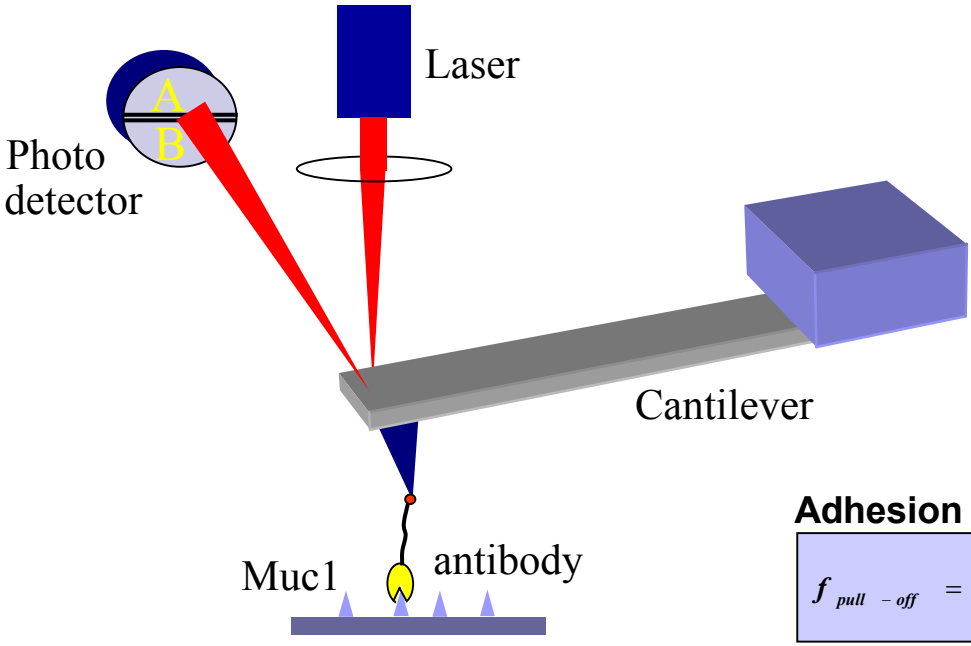
More than just imaging: Force spectroscopy for biomolecule detection or presymptomatic screening for disease

- sensitive and selective
- *in vivo* measurements
- parallel screening of targets
- high throughput capability
- label-free detection





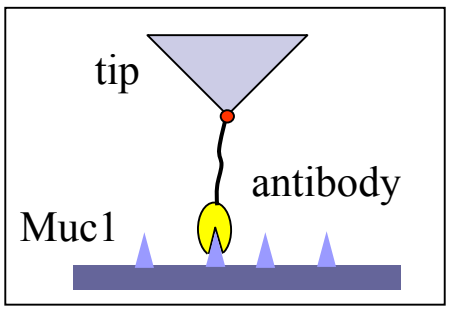
The afm provides a method to directly measure single molecule binding events



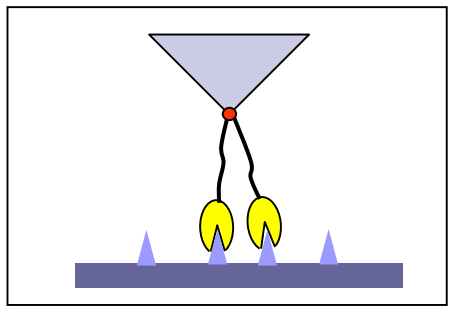
Adhesion force components

$$f_{pull-off} = \frac{\Delta H}{x_b} - T\frac{\Delta S}{x_b} - \frac{kT}{x_b} ln\left[\frac{kT}{r_f t_D x_b}\right]$$

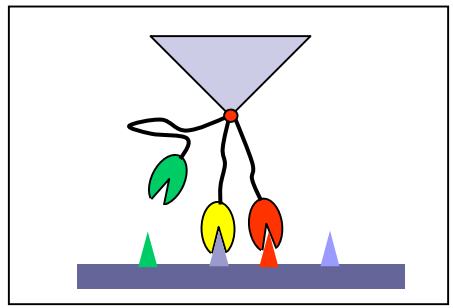
Enthalpic term | Entropic term | Thermal Activation



Single molecule binding

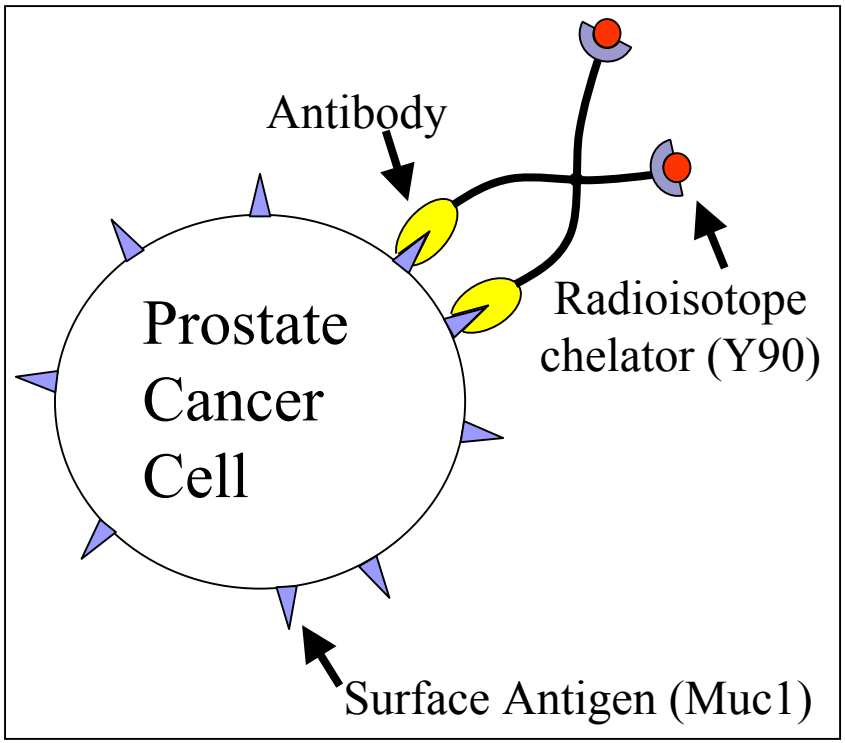


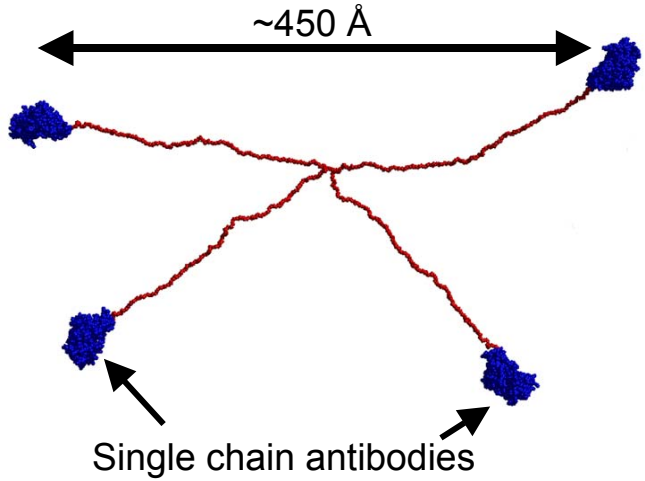
Multivalent binding

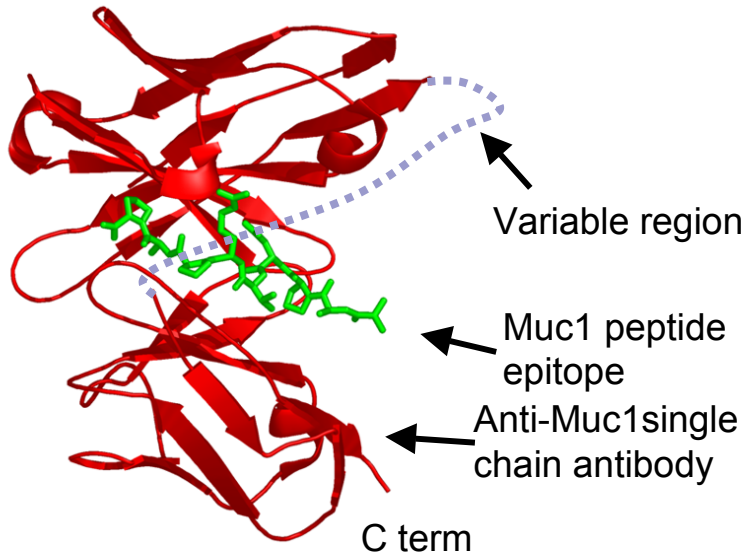


Protein complex binding

Multivalent binding is used to improve radioimmunotherapy





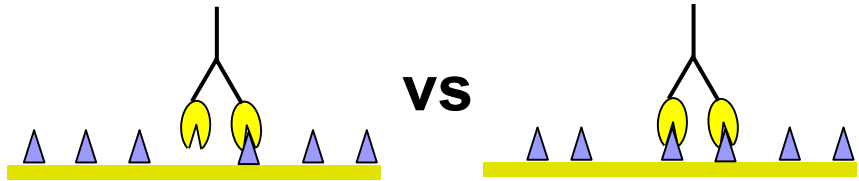


Homology model courtesy Ed Lau

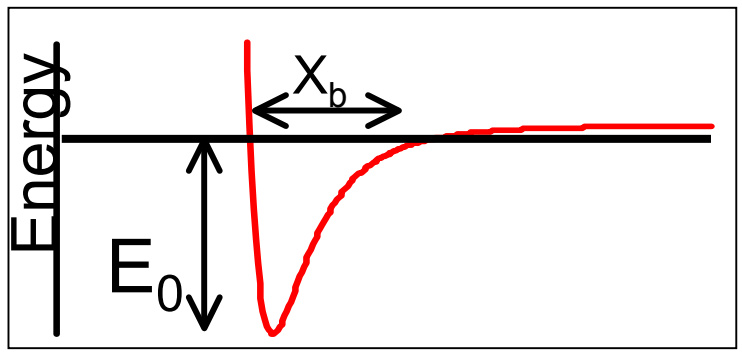
Production of ScFvs from Albrecht et al., Bioconjugate Chem. 2004, 15, 16-26.

Why are single molecule measurements useful?

We can measure characteristics of intermediate states, including binding energy magnitudes and dynamics (t_{1on}, t_{2on}).



We can measure intermolecular energy potentials.



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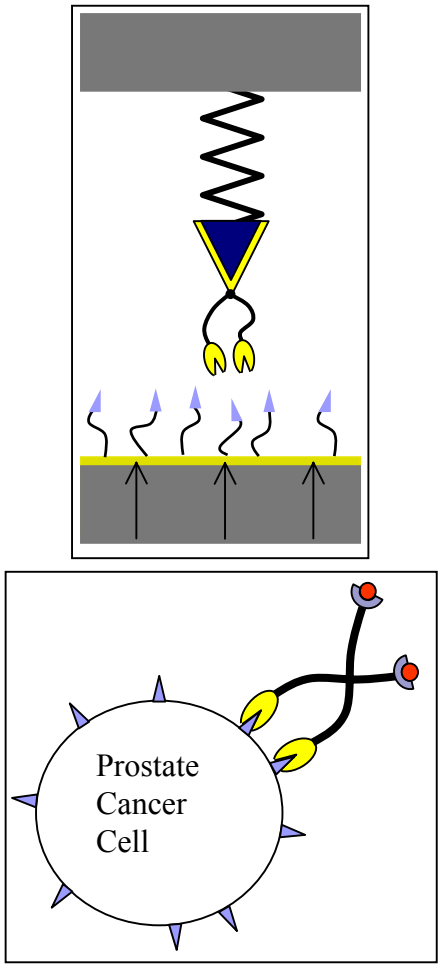
Project Goals for Muc1-antiMuc1 binding



-Directly measure the magnitude of interactions for single and multiple antibody-Mucin1 binding events

-Determine the optimal tether length for high affinity binding

- Determine the role of cooperativity

-Elucidate the kinetics of multivalent binding

-Develop a platform for identification of cancerous cells through single molecule protein-protein binding

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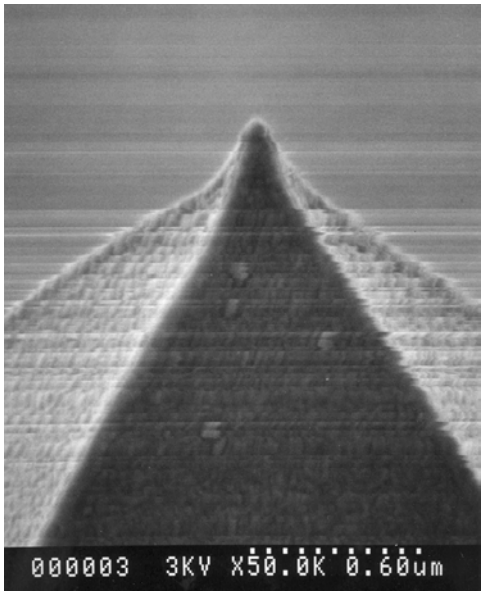
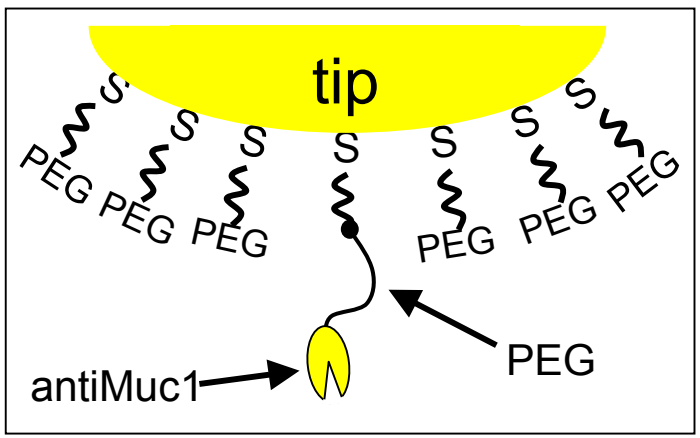
How do we functionalize the tip?





Advantage of polyethylene glycol (PEG) linkers:

a) allow orientational freedom

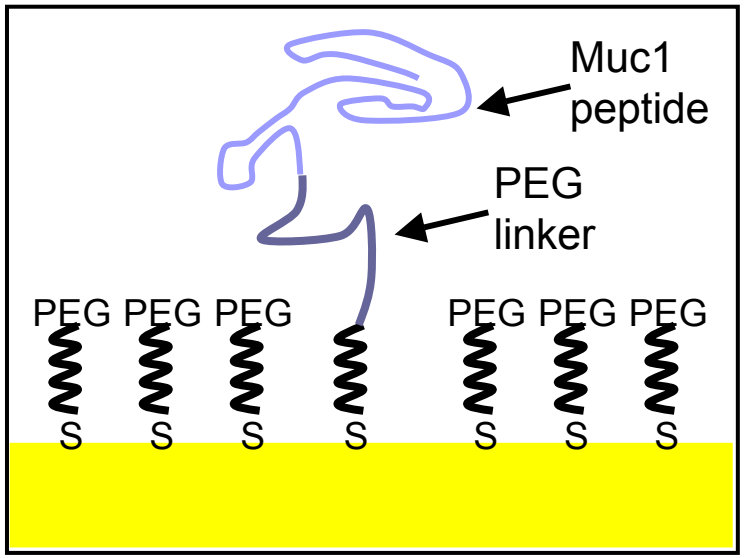
b) linkers are an integral part of the therapeutics

c) separate nonspecific tip-sample interactions from the specific interactions.

Method:

- Adsorb self assembled thioalkylamine monolayer on silicon nitride cantilevers with gold sputtered tips
- Covalently link the PEG through the amine bond. To limit the surface density of scFv, a 1:50 molar ratio of reactive PEG molecules to non-reactive methoxy-terminated PEG units .
- Functionalize the PEG tethers with the antibody fragment (~2 nM)

How do we functionalize the surface?



Method:

-The Muc1 protein core contains a 20-amino acid tandem repeat sequence, DTRPAPGSTAPPAHGVTSA (Fontenot, 1993)

-The peptide is synthesized by Synthetic Peptide Application Lab, University of Pittsburgh

-A flat gold surface is functionalized with the Muc1 peptide using the same tethering scheme developed for the cantilever tip.

-The PEG used to attach the Muc1 peptide is functional with NHS groups on each end.

- The Muc1 peptide has only one free amine group (at the N-terminus) and we use it to bind to the NHS group on the tether.

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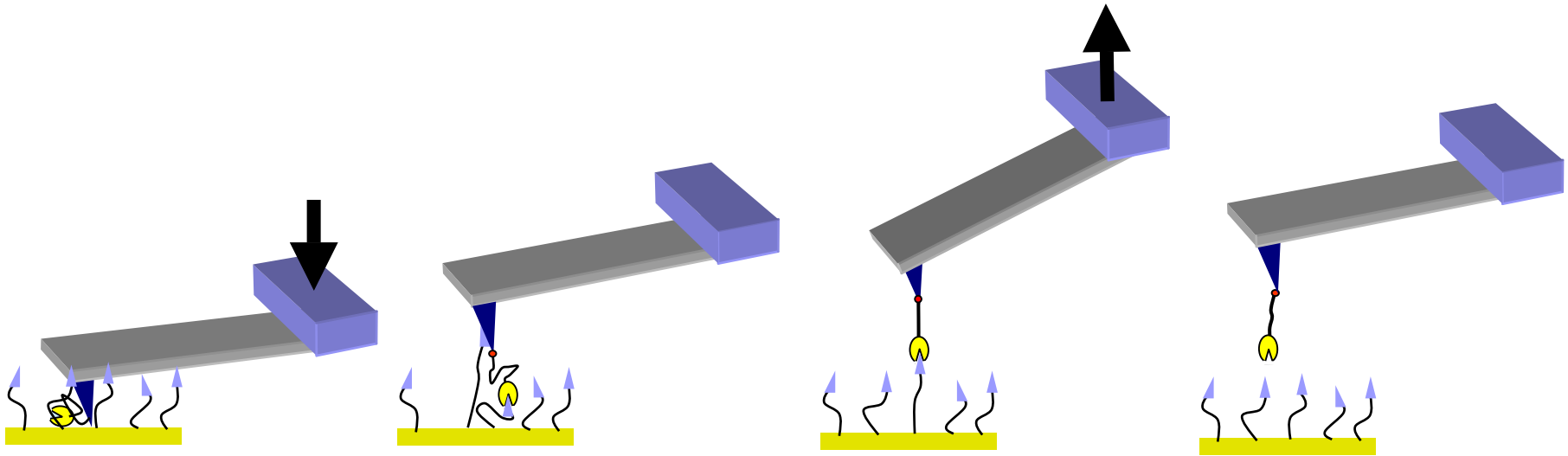
Typical AFM force curve measurements

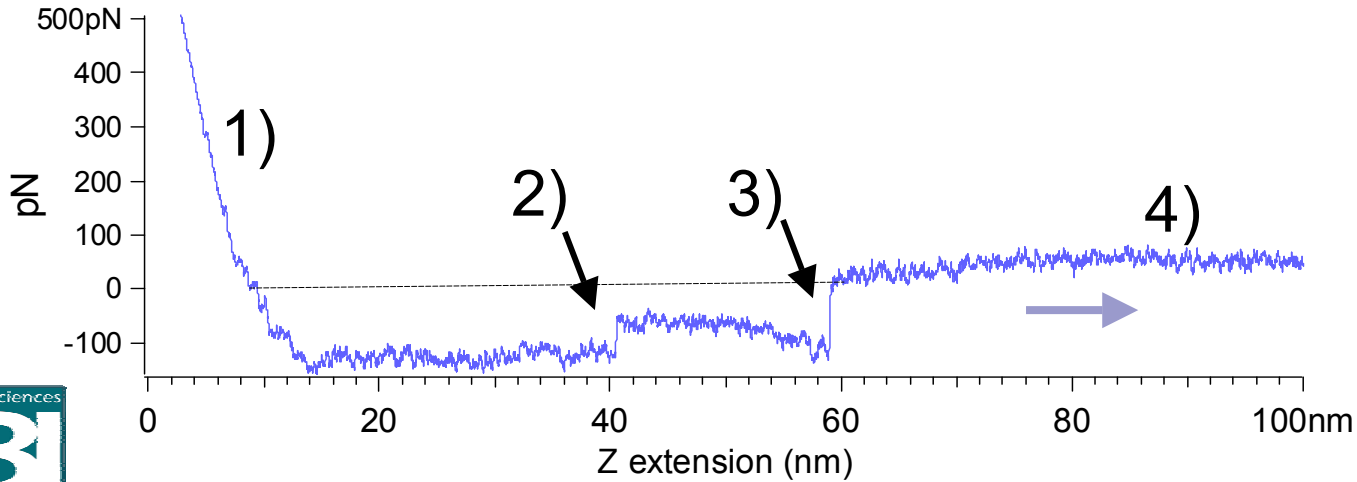


1) Repulsive tip sample interaction

2) Nonspecific binding at one tether length

3) Specific binding at two tether lengths

4) Free cantilever



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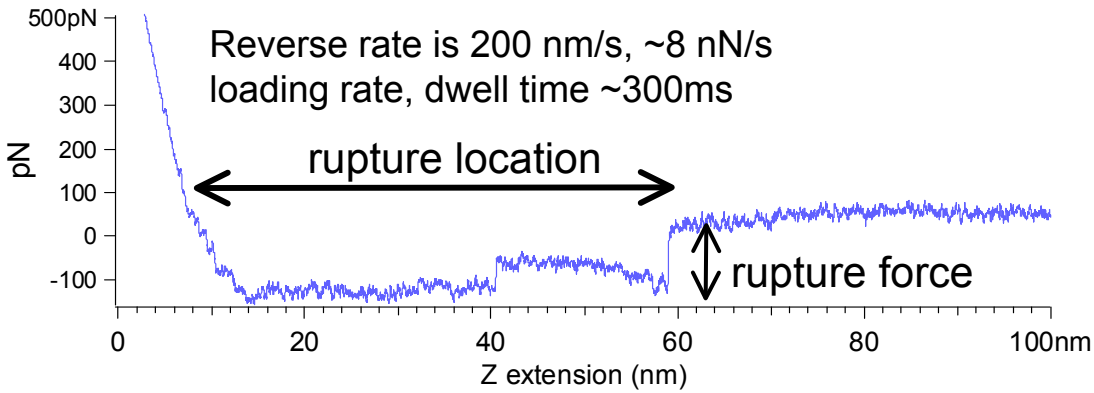
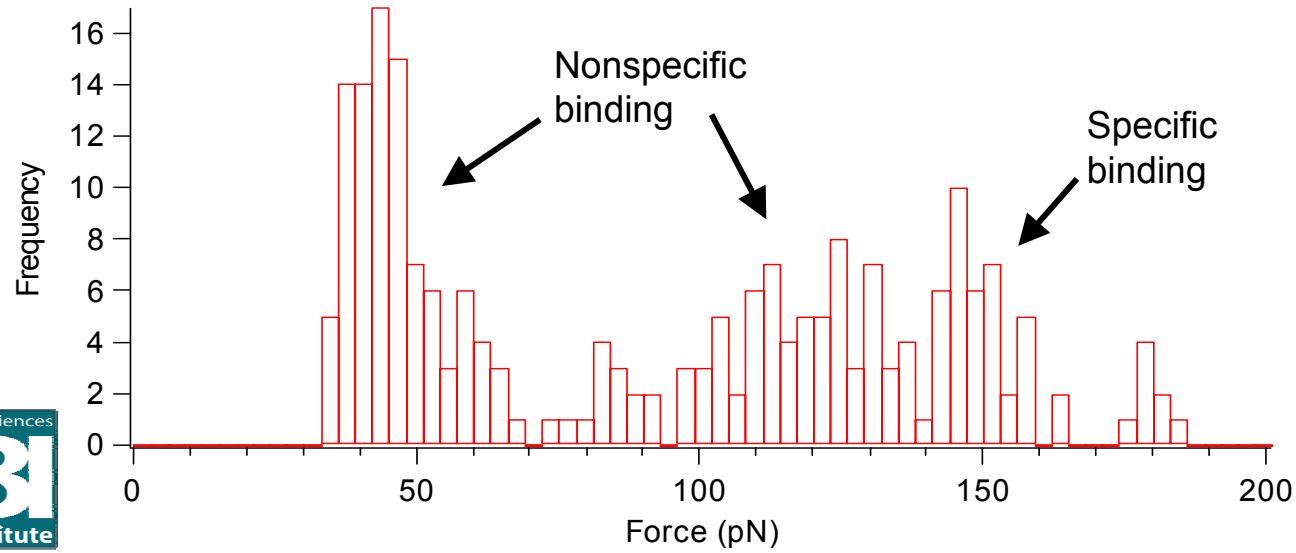
Data analysis



- We use WaveMetrics IgorPro software to automate the data analysis over thousands of force curves.

Unbinding force histogram for functionalized PEG molecules



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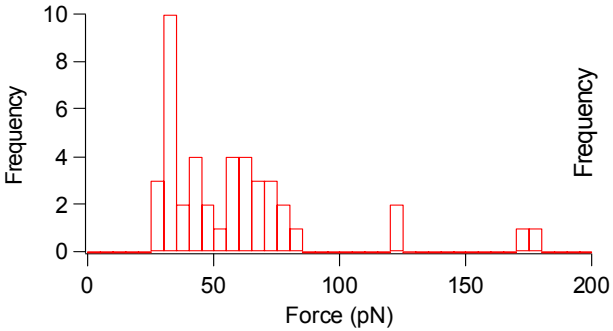
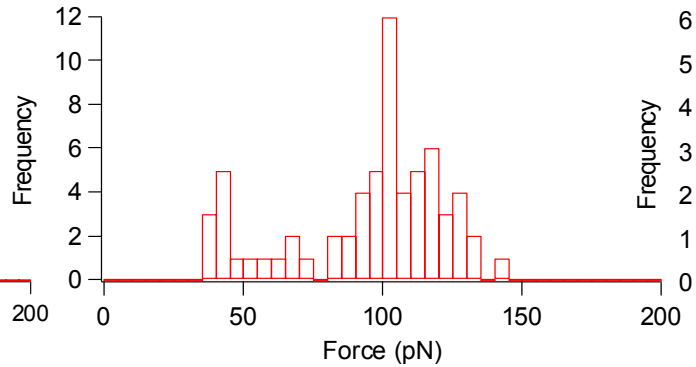
How can we identify peaks?

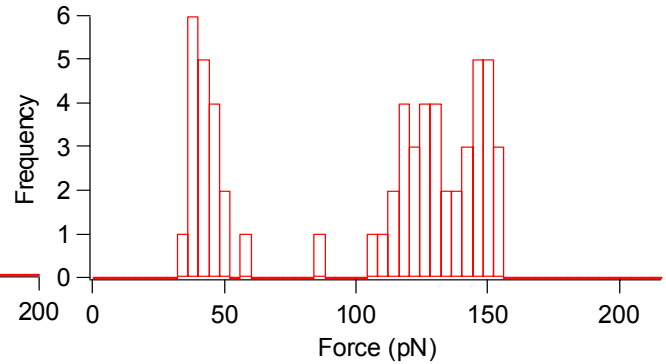
a) Force histogram for unfunctionalized PEG molecules

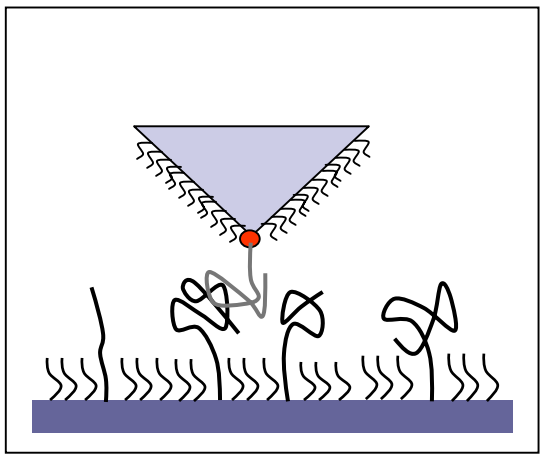


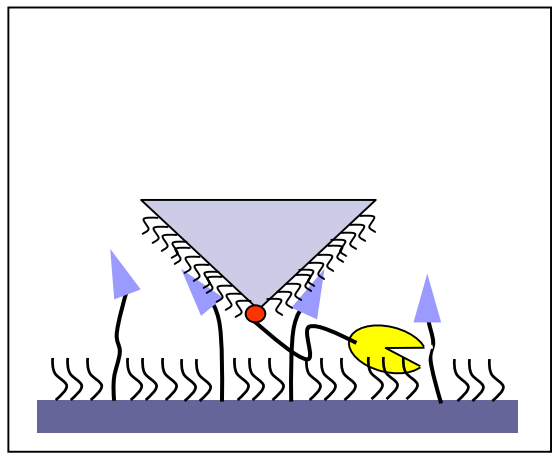
b) Unbinding force histogram for one-tether-length unbinding

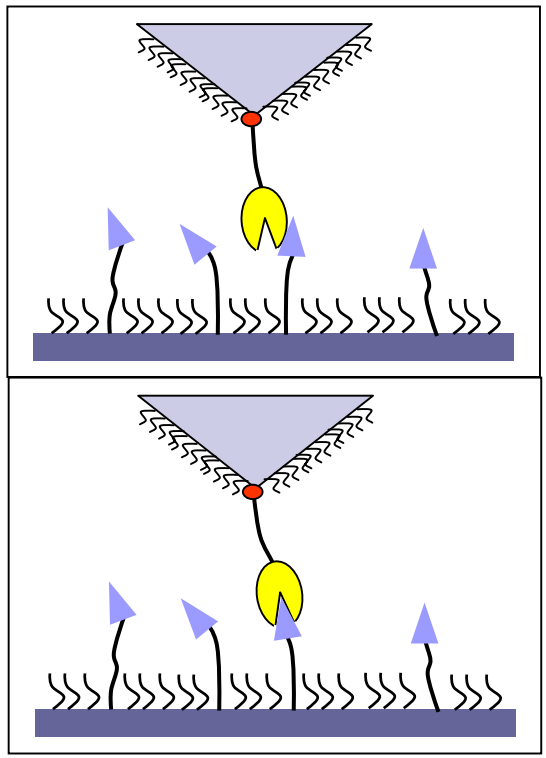


c) Unbinding force histogram for two-tether-length unbinding









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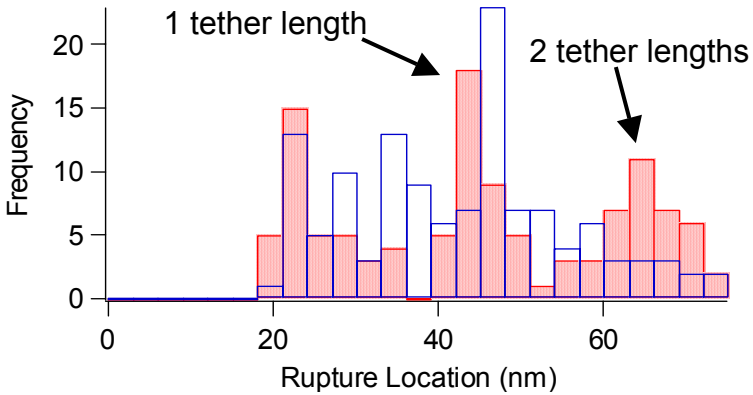
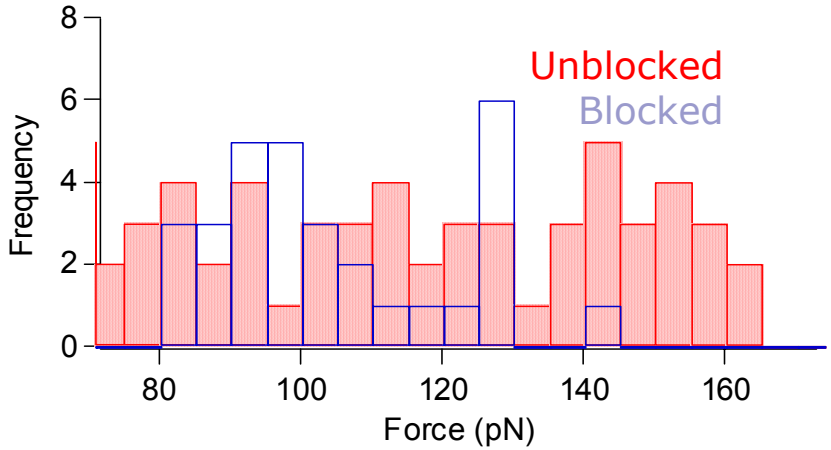
Blocking experiments

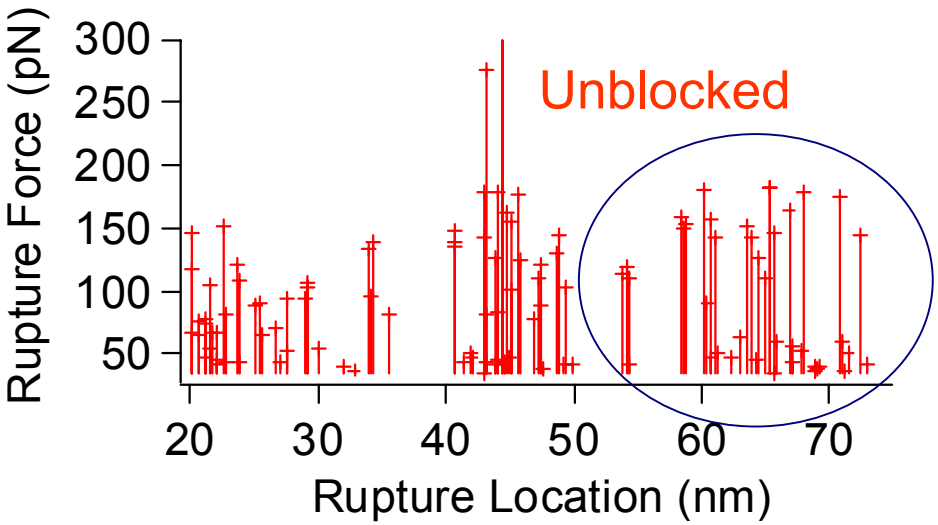
Rupture location histogram

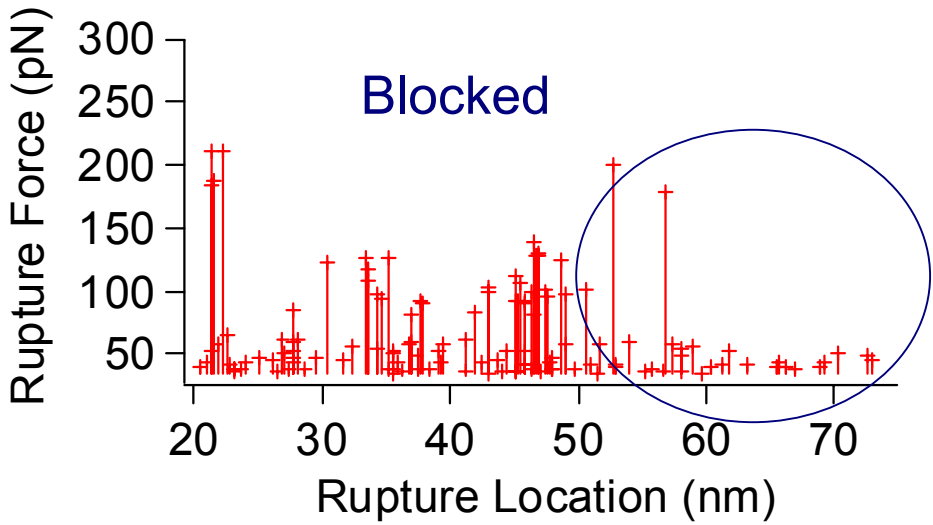


Unbinding force histogram



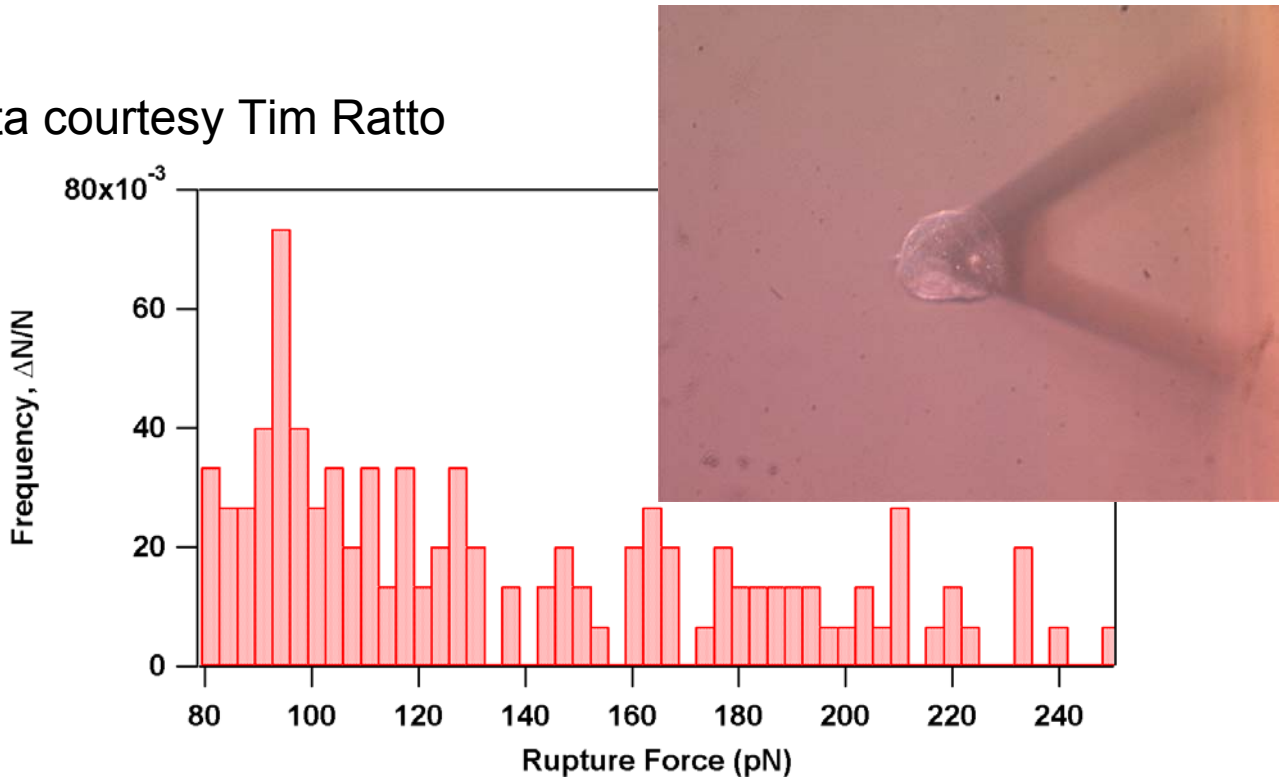
The peak at 145pN can be blocked by flowing an excess concentration free Muc1 antigen in solution (100 μM). The interactions at two tether lengths are substantially reduced by the blocking and, of those, none have a rupture force near 145 pN.





Force Spectroscopy on cancer cells

Data courtesy Tim Ratto

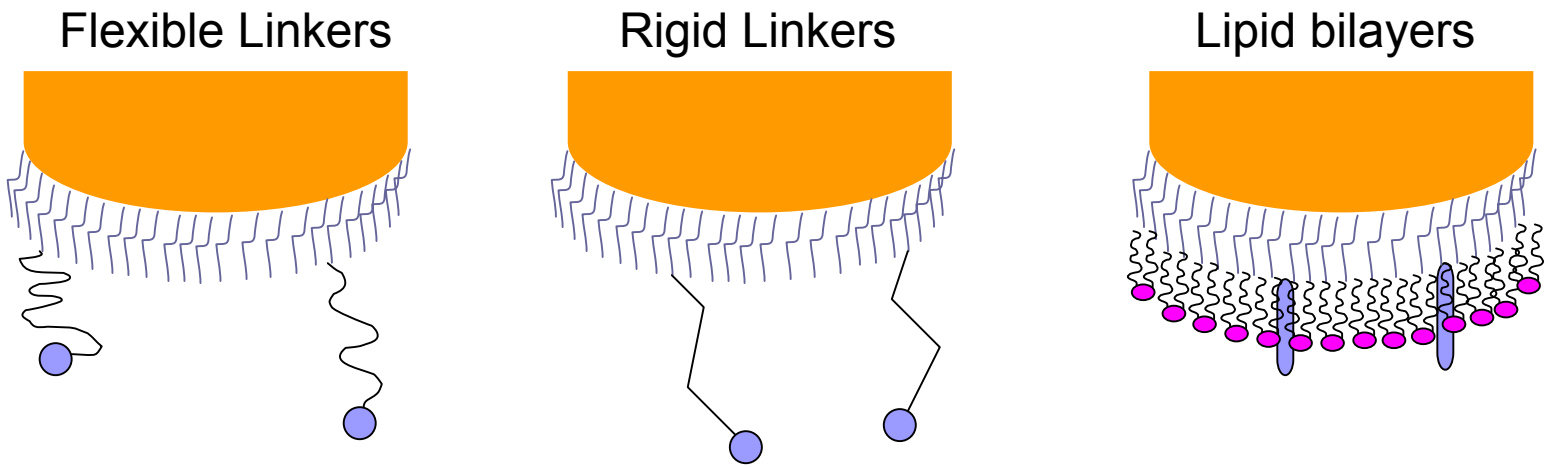


• Force histogram of the interaction between a SHAL-functionalized AFM cantilever and a raji cell, a malignant line cultured from Burkitt's lymphoma.

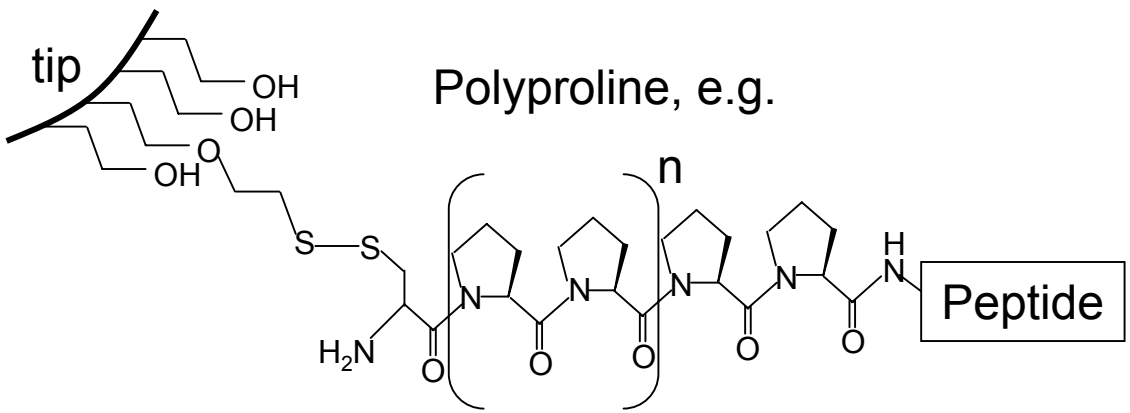
•The SHAL recognizes the HLA-Dr10 protein, a cell surface marker present on over 80% of lymphoma cells.

•Interactions are blocked by competition experiment with free HLA-Dr10

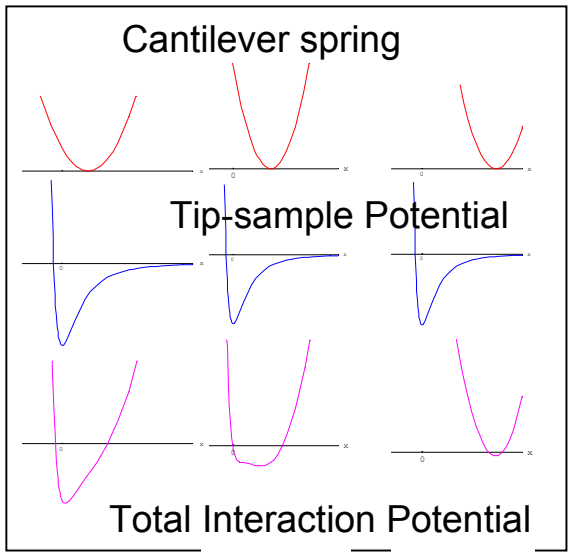
Presenting Systems: Toolkits for functionalization



- Flexible linkers allow orientational freedom
- Rigid linkers (with unsaturated bonds) assist potential reconstruction
- Lipid bilayers required to solvate membrane proteins

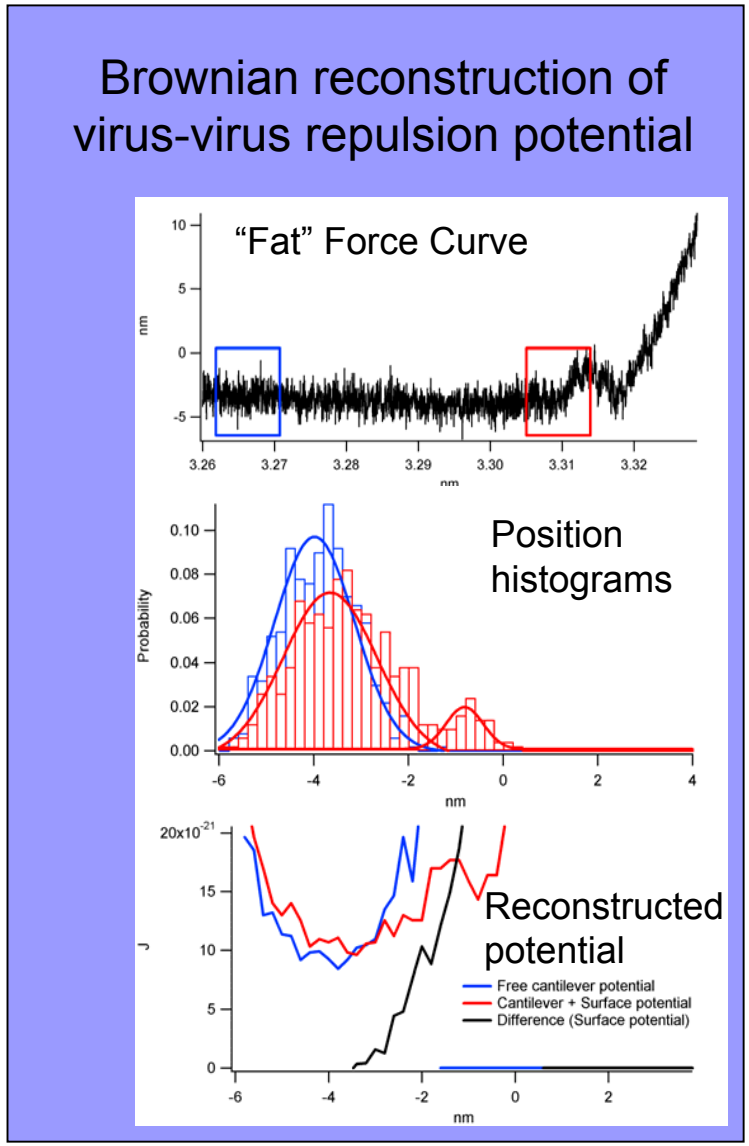


High Throughput: Brownian noise potential reconstruction



- Thermal noise of the cantilever samples total interaction potential
- Cantilever noise contains information about the tip-sample interaction potential
- Need to oversample the force curve and do *BROWNIAN RECONSTRUCTION*
- Need stiff cantilevers to avoid instabilities!
- Next generation of AFMs has the resolution and low noise to provide this information

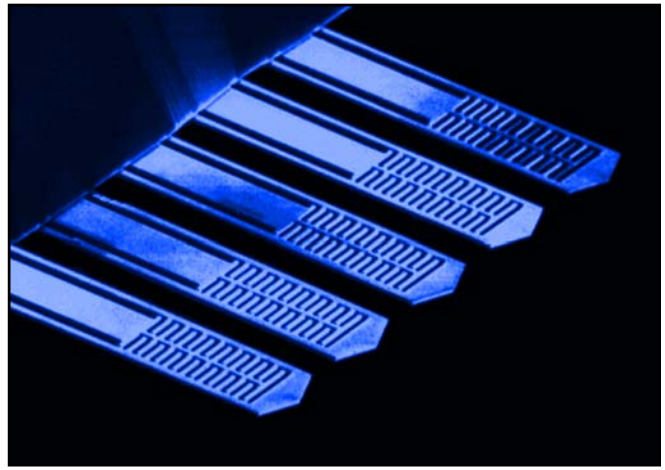
Data courtesy Alex Noy and Ray Friddle



High Throughput: Measuring multiple cantilevers in parallel

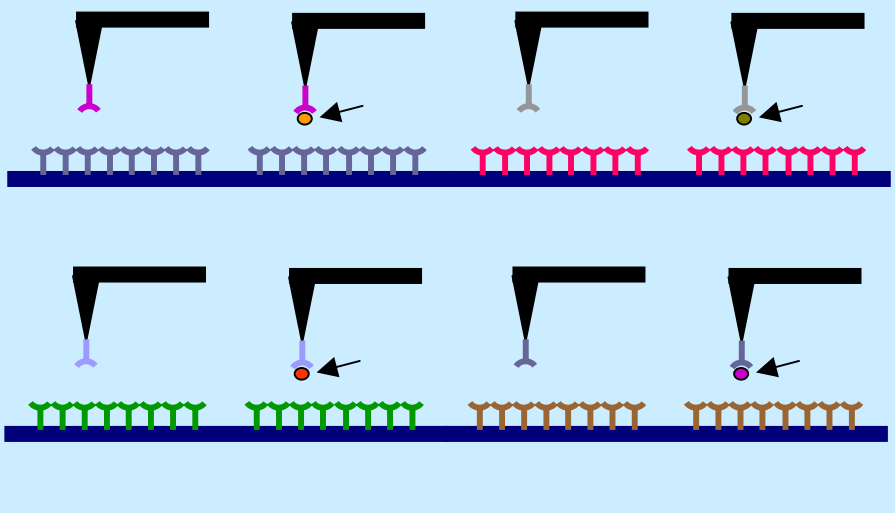
- Thousands of cantilevers can be operated in parallel, e.g. IBM's "millipede"
- Micropipettes, microfluidic, or ink jet systems to functionalize tips and samples

Interferometric sensor arrays, Stanford

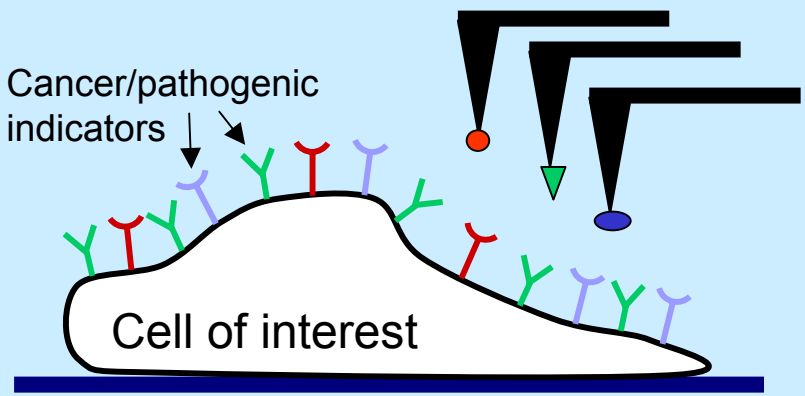


T. Sulchek, et al., Appl. Phys. Lett., 78, 1787 (2001)

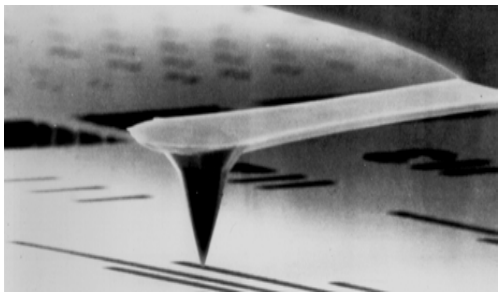
Force-based chemical and biological detection



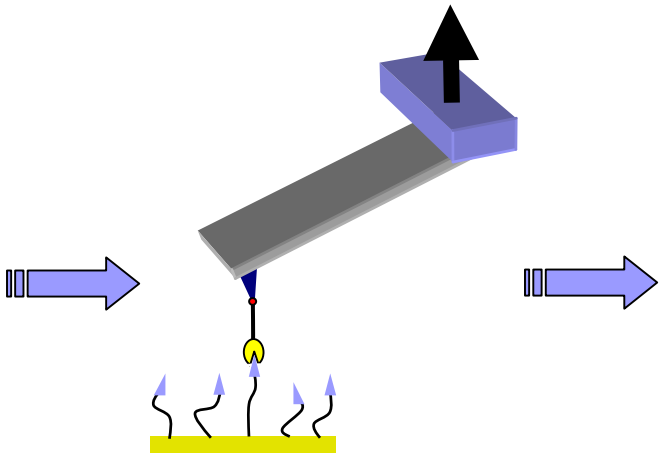
Multiple indicators increase likelihood of proper identification



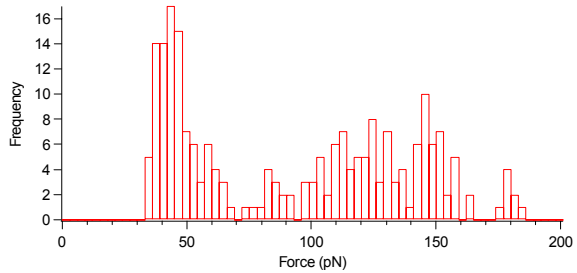
Summary of Force Spectroscopy sensing



Cantilever sensor



Force measurements



Binding signature

todds@llnl.gov

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A. Noy, S. Zepeda, C. A. Orme, Y. Yeh, J. J. De Yoreo, “Entropic Barriers in Nanoscale Adhesion,” J. Amer. Chem. Soc. 125, p 1356 (2003).

S. Zepeda, Y. Yeh, A. Noy, “Determination of Energy Barriers for Intermolecular Interactions by Variable-Temperature Chemical Force Microscopy,” Langmuir 19, p 1457 (2003).

T. V. Ratto, K. C. Langry, R. E. Rudd, R. L. Balhorn, M. W. McElfresh, “Force Spectroscopy of the Double-Tethered Concanavalin-A Mannose Bond,” Biophys J. 86, p 322A (2004).

T. Sulchek, R. J. Grow, G. G. Yaralioglu, S. C. Minne, C. F. Quate, S. R. Manalis, A. Kiraz, A. Aydine, and A. Atalar, “Parallel atomic force microscopy with optical interferometric detection,” Appl. Phys. Lett. 78, 1787 (2001).

A. Noy, D. V. Vezenov, C. M. Lieber “Chemical Force Microscopy,” Ann. Rev. Mat. Sci. 27, p 381-421 (1997) .